Retinal Arteriolar Narrowing Predicts Incidence of Diabetes

The Australian Diabetes, Obesity and Lifestyle (AusDiab) Study

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OBJECTIVE—To examine the relationship of retinal vascular caliber to incident diabetes in a population-based cohort.

RESEARCH DESIGN AND METHODS—The Australian Diabetes, Obesity and Lifestyle (AusDiab) Study recruited adults aged 25+ years across Australia in 1999–2000, with a follow-up 5 years later in 2004–2005. Participants' glycemic status was classified using fasting plasma glucose (FPG) and 2-h oral glucose tolerance (2-h plasma glucose [2hPG]) tests. Diabetes was diagnosed if FPG was ≥7.0 mmol/l or 2hPG was ≥11.1 mmol/l. Retinal vascular caliber was measured from baseline retinal photographs using a computer-assisted program.

RESULTS—Of the 803 participants without diabetes at baseline, 108 (13.4%) developed diabetes at follow-up: 7 (2.8%) of 246 participants with normal glucose tolerance, 9 (13.6%) of 66 participants with impaired fasting glucose, and 92 (18.7%) of 491 participants with impaired glucose tolerance. After multivariate analysis, participants with narrower retinal arteriolar caliber had a higher risk of diabetes (odds ratio 2.21 [95% CI 1.02–4.80], comparing smallest versus highest arteriolar caliber tertiles, P = 0.04 for trend). There was no association between retinal venular caliber and incident diabetes.

CONCLUSIONS—Narrower retinal arteriolar caliber predicted risk of diabetes. These data provide further evidence that microvascular changes may contribute to the pathogenesis of diabetes.
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The microvasculature has been hypothesized to play a key role in the pathophysiology of diabetes, insulin resistance, and related conditions such as obesity and hypertension (1–6). Retinal vascular caliber, measured quantitatively from retinal photographs, may allow an assessment of the human microcirculation in vivo. Changes in retinal caliber have now been shown to reflect early microvascular processes in pre-diabetes and diabetes (7).

Studies to date, however, have not indicated a consistent pattern of associations of retinal vascular caliber and risk of diabetes (8–10). Two previous population-based studies reported that a smaller ratio of the retinal arteriolar to venular caliber (AV ratio) was associated with incident diabetes (8,9). The significance of this observation is uncertain, since a smaller AV ratio can be due to either narrower arterioles or wider venules (11,12). The Rotterdam Study subsequently suggested that wider retinal venules may be associated with incident impaired fasting glucose but not incident diabetes (10). However, none of these previous studies defined diabetes precisely with an oral glucose tolerance test (OGTT). The purpose of our study is to examine the association of retinal vascular caliber and the 5-year incidence of diabetes in the Australian Diabetes, Obesity and Lifestyle (AusDiab) Study population.

RESEARCH DESIGN AND METHODS

The AusDiab Study included a nationally representative sample of 11,247 adults aged ≥25 years examined during 1999–2000 and 5 years later in 2004–2005. The study was approved by the International Diabetes Institute Human Ethics Committee. Informed consent was obtained from all participants (13).

All participants had an OGTT. At baseline, people identified as having diabetes, impaired fasting glucose (IFG), or impaired glucose tolerance (IGT) and a random sample of people with normal glucose tolerance (NGT) were invited to participate in a complications substudy (response rate 91% in those with diabetes and 89% in those without). Of the 1,435 without diabetes at baseline who had gradable retinal photographs (14), 803 attended the 5-year follow-up. Those who did not attend were older (mean age 57.9 vs. 56.1 years, P = 0.02) and less likely to have a family history of diabetes (19.0 vs. 24.9%, P = 0.04). Other characteristics (e.g., fasting plasma glucose [FPG], blood pressure, lipids) were not significantly different.

Definition of diabetes, IFG, IGT, and NGT. Diabetes was diagnosed as an FPG ≥7.0 mmol/l, 2-h plasma glucose (2hPG) ≥11.1 mmol/l, or treatment with insulin or oral hypoglycemic agents; IFG as FPG 6.1–6.9 mmol/l and 2hPG <7.8 mmol/l; and IGT as FPG <7.0 mmol/l and 2hPG ≥7.8 but <11.1 mmol/l per World Health Organization criteria (15). Incident diabetes was defined as participants without diabetes (NGT, IFG, or IGT) at baseline who had diabetes at follow-up.

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2hPG, 2-h plasma glucose; AusDiab, Australian Diabetes, Obesity and Lifestyle; FPG, fasting plasma glucose; IFG, impaired fasting glucose; IGT, impaired fasting glucose; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test.

TABLE 1
Participant characteristics comparing those who did and did not develop diabetes after 5 years, the Ausdiab Study

	Participants who did not develop diabetes	Participants who developed diabetes	*P*
n	695	108	
Sex (% male)	295 (42.4)	53 (49.1)	0.19
Family history of diabetes	165 (23.7)	33 (33.3)	0.04
Hypertension present	306 (44.2)	59 (55.7)	0.08
Hypertension medication	142 (20.5)	26 (24.5)	0.55
Lipid-lowering medication	78 (11.2)	17 (15.7)	0.13
Physical activity (≥150 min)	308 (44.6)	46 (43.4)	0.81
Completed secondary schooling	324 (46.7)	41 (38.7)	0.12
Glucose tolerance status			<0.001
NGT	239 (34.4)	7 (6.5)	
IGT	399 (57.4)	92 (85.2)	
IFG	57 (8.2)	9 (8.3)	
Smoking status			0.08
Current	60 (8.8)	13 (12.4)	
Ex-smoker	213 (31.2)	41 (39.0)	
Never smoked	409 (60.0)	51 (48.6)	
Retinopathy	23 (3.1)	5 (4.0)	0.64
Age (years)	55.9 ± 12.9	57.7 ± 11.3	0.17
FPG (mmol/l)	5.55 ± 0.55	5.99 ± 0.59	<0.001
Systolic blood pressure (mmHg)	133.5 ± 18.8	136.0 ± 16.8	0.19
Diastolic blood pressure (mmHg)	70.5 ± 11.5	74.3 ± 12.4	0.002
BMI (kg/m^2)	27.5 ± 5.0	30.2 ± 6.1	<0.001
Waist circumference (cm)	92.1 ± 13.2	98.9 ± 15.2	<0.001
HDL cholesterol (mmol/l)	1.45 ± 0.39	1.31 ± 0.46	0.001
Triglycerides (mmol/l)	1.50 ± 1.00	2.14 ± 1.62	<0.001
Microalbumin-to-creatinine ratio (mg/mmol)	1.82 ± 6.44	2.45 ± 9.08	0.38
Fasting insulin (μ/ml)	15.0 ± 9.8	19.4 ±11.5	<0.001

Data are *n* (%) and means ± SD.

Measurement of retinal vascular caliber and assessment of retinopathy. Nonmydriatic digital retinal photographs were taken at baseline (14). Retinal vascular caliber was measured using a validated computer-based program by trained graders masked to participant characteristics (16). If retinal vascular caliber could not be measured from photographs of the right eye, the left was used. For each photograph, the average arteriolar and venular caliber was summarized as central retinal artery equivalent and central retinal vein equivalent, respectively (17). Reproducibility of this method was high, with intra- and intergrader intraclass correlation coefficients 0.78–0.99 (17).

Retinopathy was graded from photographs by one trained assessor. The level of retinopathy was defined according to a simplified version of the Wisconsin grading system (14). Retinopathy was defined as the presence of at least one definite retinal hemorrhage and or microaneurysm, with the classification of the subject based on the grading of the worst eye. A random sample of 167 retinal photographs (with and without retinopathy) were regraded (by the same assessor), and the intra-observer agreement was high (unweighted $\kappa = 0.732$) (14).

Other risk factors. Family history of diabetes was defined as mother or father with diabetes. Fasting HDL and triglycerides were determined by enzymatic methods. Insulin analysis was conducted using the Human Insulin Specific RIA Kit. Urine protein and creatinine (microalbumin-to-creatinine ratio) were measured on a morning spot urine sample by enzymatic method. Blood pressure was measured in the supine position from the right arm using standard mercury sphygmomanometer after 10 min of resting. BMI was calculated as weight in kilograms divided by the square of height in meters. Hypertension was defined as blood pressure ≥140/90 mmHg or current use of antihypertensive medication. Education was classified into two categories: under or beyond secondary school completion level. Total physical activity time was calculated as the sum of the time spent performing moderate activity (including walking) plus double the time spent in vigorous activity and categorized as inactive/insufficient (<149 min of physical activity in the previous week) and sufficient (at least 150 min of physical activity). Smoking status was defined as current smoker if smoking tobacco products at least daily, ex-smoker if previously smoked daily, and never smoker if never smoked daily.

Statistical analysis. Retinal vascular caliber was categorized into tertiles for analysis. Logistic regression models were used to determine the risk of diabetes in association with retinal vascular caliber. We constructed three models. Model 1 was adjusted for age, sex, and venular caliber (in models of arteriolar caliber) or arteriolar caliber (in models of venular) to control for potential confounding of the two vessel calibers (18); model 2 was further adjusted for FPG, systolic blood pressure, family history of diabetes, waist circumference, HDL cholesterol, triglycerides, smoking status, and retinopathy; and model 3 was adjusted for variables in model 2 plus other potential confounders: BMI, presence of hypertension (instead of systolic blood pressure), glucose tolerance status (NGT vs. IFG/IGT), physical activity, education level, microalbumin-to-creatinine ratio, and fasting insulin concentration. All analyses were performed in SPSS version 12.0.1 (SPSS, Chicago, IL).

RESULTS

Of the 803 participants included in this study, 246 (30.6%) had NGT, 66 (8.2%) had IFG, and 491 (61.1%) had IGT. After a median follow-up time of 4.98 years (interquartile range 0.10 years), 108 participants (13.4%) developed diabetes at follow-up: 7 (2.8%) from NGT, 9 (13.6%) from IFG, and 92 (18.7%) from IGT. Participants who developed diabetes were more likely to have impaired glucose metabolism and had significantly higher mean FPG, higher diastolic blood pressure, higher BMI, higher fasting insulin levels, larger waist circumference, lower HDL cholesterol, and higher triglycerides at baseline. However, hypertension status, use of hypertensive or lipid-lowering medications, level of physical activity, level of education, microalbumin-to-creatinine ratio, smoking status, and retinopathy were not significantly different between the two groups (Table 1).

Participants with narrower retinal arteriolar caliber had higher risk of diabetes (Table 2). This persisted after adjusting for age, sex, retinal venular caliber, FPG, systolic

TABLE 2
Retinal vascular caliber and 5-year risk of diabetes

	Number at risk	Incident diabetes			
		Number (%) events	Model 1*	Model 2†	Model 3‡
Retinal arteriolar caliber					
Tertile 1: ≤167 μm	254	42 (16.5)	2.14 (1.13–4.05)	2.37 (1.14–4.91)	2.21 (1.02–4.80)
Tertile 2: 167–188 μm	301	41 (13.6)	1.50 (0.86–2.60)	1.43 (0.75–2.72)	1.34 (0.66–2.69)
Tertile 3: ≥188 μm	248	25 (10.1)	1.00	1.00	1.00
P for trend			0.02	0.02	0.04
Retinal venular caliber					
Tertile 1: ≤196 μm	264	38 (14.4)	1.00	1.00	1.00
Tertile 2: 196–216 μm	287	39 (13.6)	1.10 (0.65–1.84)	0.71 (0.39–1.30)	0.78 (0.42–1.47)
Tertile 3: ≥216 μm	252	31 (12.3)	1.11 (0.61–2.01)	0.96 (0.45–1.73)	0.82 (0.40–1.69)
P for trend			0.74	0.70	0.58

Data are OR (95% CI) unless otherwise indicated. *Adjusted for age, sex, and venular caliber (in models of arteriolar caliber) or arteriolar caliber (in models of venular caliber). †Adjusted for variables in model 1 plus FPG, systolic blood pressure, family history of diabetes, waist circumference, HDL cholesterol, triglycerides, smoking status, and retinopathy. ‡Adjusted for variables in model 2 plus BMI, presence of hypertension (instead of systolic blood pressure), glucose tolerance status (NGT vs. IFG/IGT), physical activity, education level, microalbumin-to-creatinine ratio, and fasting insulin concentration.

blood pressure, family history of diabetes, waist circumference, HDL cholesterol, triglycerides, smoking status, and retinopathy (OR 2.37 for smallest vs. highest tertiles, model 2). With further adjusting for other potentially important confounding factors (model 3), BMI, presence of hypertension (instead of systolic blood pressure), glucose tolerance status (NGT vs. IFG/IGT), physical activity, education level, microalbumin-to-creatinine ratio, and fasting insulin concentration, the association between narrower arteriolar caliber and incident diabetes remained (OR 2.21 [95% CI 1.02–4.80] for smallest vs. highest tertiles). There was no association between retinal venules and incident diabetes.

DISCUSSION

In this prospective cohort of adult Australians, narrower retinal arterioles were associated with increased risk of developing diabetes, independent of FPG, systolic blood pressure, family history of diabetes, waist circumference, and other factors. Retinal venular caliber was not associated with risk of diabetes.

Our study provides further evidence to support the hypothesis that changes in the microvasculature may precede the development of diabetes (1–6,19–21). Previous studies that examined the relationship of retinal vascular caliber and diabetes were limited by lack of an OGTT to precisely classify diabetes status (8–10). Some studies also did not clarify whether arteriolar or venular caliber was associated with diabetes risk (8,9). Our data show that narrower arterioles, but not wider venules, are associated with an increased diabetes risk, suggesting that arteriolar processes may play a role in the pathogenesis of diabetes. This finding contrasts with our baseline cross-sectional analytical finding of wider arterioles with diabetes (16).

There are several biological mechanisms that may explain the observed association. Previous studies have indicated the presence of functional microcirculatory changes in individuals at high risk of developing diabetes (1–3). It is recognized that there are dual insulin signaling mechanisms in blood vessel autoregulation—stimulation of nitric oxide (NO, a vasodilator) synthesis and release of endothelin-1 (ET-1, a vasoconstrictor) (22). Insulin-induced vasoconstriction (19) together with increased ET-1–dependent vasoconstrictor tone and decreased NO-dependent vasodilator tone in resistance arteries (20) have been demonstrated in obese, hypertensive individuals. Endothelial dysfunction and impaired NO-mediated vasodilatation are further hypothesized to reduce insulin delivery to skeletal muscles, resulting in peripheral insulin resistance and hyperglycemia (21). New studies further suggest that systemic markers of endothelial dysfunction and inflammation predict the development of diabetes (23–25). It is therefore possible that the association of narrower retinal arterioles and incident diabetes we observed here reflects both structural microvascular changes and pathophysiological processes related to endothelial dysfunction and inflammation.

Strengths of our study include its population-based sample, use of a validated computer software program to measure retinal vascular caliber, and classification of diabetes using OGTT. Study limitations include the possibility of selection biases, as only 56.7% of participants without diabetes at baseline attended follow-up, and the inability to analyze associations separately for NGT, IFG, and IGT due to limited sample size.

In conclusion, we found that narrower retinal arteriolar caliber predicted a doubling of the risk of diabetes, independent of other diabetes risk factors. This finding suggests that retinal arteriolar narrowing might be a subclinical marker of diabetes risk and provides further evidence that early microvascular changes may contribute to the pathophysiology of diabetes development (1–6,19–21).

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